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Bioconversion of Agro Forest Residues for Production of Oyster Mushrooms

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Abstract— *The oyster mushroom (Pleurotus sp.)is the third most cultivated edible mushroom in the cultivated in the temperate and sub tropical regions of the world. Recently, cultivation of Pleurotus spp. is gaining popularity as an income generating enterprise in our country. Normally wheat straw and paddy straw are used as substrates for cultivation of oyster mushroom. Very often availability of these substrates becomes a limiting factor as its used as main cattle feed.Mushroom mycelia secrete large amounts of extracellular enzymes that break down compounds such as cellulose and lignin present in the substrate. With this in view the lignocellulolytic enzymes of Pleurotus florida were exploited for degradation of agro residues like Teak, Populus, Eucalyptus, Bamboo . TheiIn vitro studies in liquid medium(PDA broth) revealed that leaf litter from Teak, Bamboo, Populus, Eucalyptus in combination with paddy straw and paddy straw substrate alone supported good mycelial growth with 0.15g, 0.18 g, 0.2g, 0.18 g and 0.19 g respectively. Among the substrates tested for cultivation of oyster mushroom , teak leaf litter + paddy straw substrate and paddy straw + combination of all substrates (Eucalyptus, Populus, Teak, Bamboo , paddy straw) supported early spawn running (DFSR) (16.67 days) followed by paddy straw (18.67 days). The DFSR was delayed in Paddy straw+ Bamboo, Paddy straw+ Populus, Paddy straw+ Eucalyptus (19.67, 20.0, 20.33 days respectively). The days for pin head formation was early in teak + paddy straw substrate and paddy straw + combination of all substrates (18.0 and 18.67 days) compared to other substrates. Also the days for first harvest (DFFH) started on 20th day in teak + paddy straw substrate and paddy straw + combination of all substrates (20.0 and 20.67 days respectively) itself compared to other substrates which took around 23-24 days. The yield was significantly higher in paddy straw substrate (470g/500g substrate; 94% bio efficiency) followed by paddy straw+ combination of all substrates (426.67g/500 g substrate; 85.3 % bio efficiency).Outdoor cultivation of oyster mushroomP.florida and P.eous var.APK- 1in bamboo plantations proved successful with maximum yield in paddy straw substrate (450 g/ bed: 90 % bioefficiency) followed by paddy straw+ bamboo substrate (400g; 80 % bioefficiency). It is well understood that oyster mushroom cultivation will certainly fit best in agroforestry model and the concept of integrating mushroom cultivation with growing of trees can provide additional income and serve the family with protein rich pharmacological rich food.*

Keywords— Degradation, agroforest residues, cultivation, oyster mushroom.

I. INTRODUCTION

Biotechnological process through recycling of lignocellulosic organic waste for cultivation of edible mushrooms leads to production of protein-rich food combined with the reduction of environmental pollution (Sánchez, 2010); Mandeel et. al., 2010). As a pioneer, since 1970's the Tamil Nadu Agricultural University (TNAU), Coimbatore was the first to lay pathway to popularize cultivation technology of oyster mushroom ,paddy straw mushroom and in 21st century for milky mushroom ,shitake mushroom, Jews mushroom , Black poplar mushroom and caterpillar mushroom. However, due to the training efforts made by TNAU has led to expansion of several medium scale mushroom growers who produce oyster mushrooms targeting big city markets and this has made Tamil Nadu state the leading producer of oyster mushrooms in India. *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures and need a short growth time and their fruiting bodies are not often attacked by diseases and pests.(Tesfaw *et. al*., 2015).

It is estimated that about 947 MT of agroresidues and 204 MT of forest related wastes are being produced in our country (Agricultural waste management Policy paper 49 NAAS, Dec 2010) and at present only 0.04 % of this is used

to produce mushrooms contributing to 3% of total world mushroom production .Due to the climate change scenario in Tamil Nadu and due to lack of sufficient rainfall and water scarcity, the area under paddy has decreased which has resulted in shortage of paddy straw. Moreover, the paddy straw has to be transported for long distances that led to increased cost of the substrate. However, the interest on oyster mushroom is increasing largely due to its taste, nutrient, and medicinal properties .Hence it was felt worthwhile to educate the farmers to utilize the agroforest residues for cultivation of oyster mushroom.

In the recent days, farmers have shown *great* interest in establishing tree plantations like Casuarina, Eucalyptus, Melia, Teak, Khaya, and Sisoo that fetch high returns. However, the tree residues, especially leaves are left in the plantation uncared and burnt in situ that creates environmental pollution. In this context utilization of tree residues for cultivation of oyster mushroom by partial substitution with paddy straw was thought of as it would reduce the cost of cultivation apart from bioconversion of leaf litter in to protein rich food. In general, agro wastes consist of lignin, cellulose and hemicellulose in different ratio. During the process of degradation, the extracellular lignolytic enzymes like lignin peroxidase, manganese peroxidase and laccases, act on the lignin and convert in to simpler molecules (Leatham, 1985). Widely used substrates for oyster mushroom cultivation include rice straw, wheat straw and sawdust (Chang, 1999).However, several workers have made efforts to exploit the potential of various wastes for commercial production of oyster mushrooms *viz*., cereal straw, paddy straw (Jandaik and Kapoor, 1974; Khanna and Garcha, 1982),wheat straw (Bano and Rajarathnam, 1982; Thampi et. al., 1996). Sorghum/ maize straw to cultivate *P. sajor-caju* (Bahukhandi and Munjal, 1989), Rye straw waste (Pal and Thapa, 1979), lawn grass (Yamashita *et al*., 1983), spent brewery grain in combination with wheat bran (Andrej Gregory,2008), elephant grass (Obodai et al., 2003), coffee husk (Gume *et. al*., 2013) maize cobs and Banana waste (Bonatti et al., 2004) Grevillea leaves (Fekadu Alemu, 2014) were reported as suitable substrates for cultivations as alternative substrates for oyster mushroom cultivation. Similarly, Diwakar et. al.(1989) reported the usefulness of pearl millet stalks in the cultivation of *P. sajor-caju*.. Bhandari *et. al*.(1991) successfully cultivated *P. sajor-caju* on straws of *Echinochloa frumentacea* and *Eleusine coracana*, *Heteropogon contortus* and *Andropogon purtuses*..However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates.In open field cultivation of oyster mushroom conditions in bamboo plantations at Tanjore revealed that the oyster mushrooms *Pleurotus florida* followed by *P.djamor roseus, P.platypus* recorded about 90 to 100 % bioefficiency both in paddy straw substrate and in bamboo leaf substrate (Prakasam *et.al.*, 2013).

It is need of the hour to address the nutritional security of the growing younger generation and to provide self sustained employment opportunities for which mushroom cultivation is one of the best enterprise that can improve and secure environment, nutrition and economical status of the society and promote organic agriculture.

With this in view, the present study was undertaken to utilize various forest tree residues for cultivation of oyster mushroom and to cultivate mushrooms under natural conditions in forest ecosystem.

II. MATERIALS AND METHODS

2.1 Isolation of pure culture of *Pleurotus florida* **:**The mushroom *Pleurotus florida* was obtained from Department of Plant Pathology, TNAU, Coimbatore and used for isolation using tissue culture technique in Potato Dextrose Agar medium under aseptic conditions .The pure culture of *P.florida* observed as whitish mycelial growth was transferred to PDA slants and used for further studies.

2.2 Selection of substrates used for the study

The forest tree residues mainly dried leaves from Teak (*Tectona grandis*), Cadamba (*Anthocepahalus cadamba*) , Casuarina (*Casuarina equisettifolia*), Bamboo (*Bambusa bamboos*) , Sisoo (*Dalbergia sisoo*) , Khaya (*Khayas enegalensis*) , Pungam (*Derris indica*) , Populus (*Populus deltoides*),Eucalyptus (*Eucalyptus teretticornis*) were selected. Paddy straw substrate was used for comparison.

2.3 In vitro screening of mycelial growth of P.florida in forest tree leaf substrates (liquid medium)

PDA broth (without agar) 30 ml was prepared in 100 ml conical flasks .and to this 1 % of tree substrates *viz*, powdered leaf wastes of Teak ,Cadamba , Casuarina , Bamboo, Sisoo, Khaya , Pungam, Populus , Eucalyptus and Paddy straw were added and the contents were autoclaved under 15 psi for 20 minutes. A 9 mm mycelial disc of *Pleurotus florida* was inoculated and incubated for 7 days under room temperature.

2.4 *In vitro* mycelial growth of *P.florida* on selected substrates in combination with paddy straw (liquid medium)

Based on the above results, powdered samples from leaves of Teak ,Cadamba , Casuarina , Bamboo, Sisoo, Khaya ,Pungam, Populus , Eucalyptus were added to paddy straw powder @ 0.5% in 30 ml PDA broth prepared in 100 ml

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conical flasks under aseptic conditions . A 9 mm mycelial disc of *Pleurotus florida* was inoculated and incubated for 7 days under room temperature. The mycelial growth was recorded on 7th day after inoculation and the mycelial dry weight was recorded.

2.5 Cultivation of oyster mushroom Pleurotus florida in selected tree substrates in cropping rooms

The sorghum based spawn of *P.florida* was obtained from Department of Plant Pathology, TNAU, Coimbatore and used for the studies. Thatched shed was used for cropping of oyster mushroom *P.florida*. Sand was filled to 1 feet height at the bottom of the floor and kept wet with water to maintain humidity of 75 to 80 % with temperature of 25 to 28 °C.

Based on the results obtained from above studies, the substrates *viz*., Teak, Eucalyptus, Bamboo and Populus were included for the study.The dried leaf substrates from Teak, Eucalyptus, Bamboo and Populus were collected from the plantations of Forest College and Research Institute, Mettupalayam . The leaf substrates were combined with chopped paddy straw @ 1: 1 ratio and used for the study. The substrates were soaked in water for 30 min and steamed in autoclave for 1 h. Later the substrates were drained and dried in shade until 60 % moisture. Cylindrical beds were prepared by combining paddy straw and respective substrates 1:1 (dry weight 500g substrate/ bed) using spawn of *Pleurotus florida*. Paddy straw substrate alone served as control. Eight to ten holes were made in cylindrical beds and the beds were placed at room temperature of 27 to 30 °C . Later, after spawn running (18 – 20 days) the beds were fully covered with whitish mycelial growth and transferred to cropping rooms (thatched) which was maintained at 25 to 28° C with relative humidity of 75 to 80%. At this pin head appearance stage, water was sprinkled daily over the beds. The days for Spawn run (DFSR), Days for pin head formation (DFPF), Days for first harvest (DFFH), yield and biological efficiency (%) were recorded in different treatments. The experiments were laid out in completely randomized block design (CRD) with three replications. Statistical software (AGRES) was used for the analysis of the data

$$\text{Biological efficiency (\%)} = \frac{\text{Total weight of mushrooms harvested (in grams)}}{\text{Total quantity of paddy straw used (in grams)}} X100$$

2.6 Out door cultivation of oyster mushroom in bamboo plantations

Bamboo plantations normally maintain cool and humid climate(25 to 28° C ;relative humidity of 75 to 80%) which is quite suitable for oyster mushroom cultivation. Could be exploited for cultivation of outdoor cultivation of oyster mushroom. For this, the mushroom *Pleurotus florida* var. PF and *Pleurotuseous*var.APK -1 was selected for the studies. The substrates bamboo ,paddy straw and paddy straw + bamboo litter were included for the study. Cylindrical beds (500g dry weight / bed) were prepared using the spawn of *P.eous* var.APK- 1 and *P.florida* as mentioned earlier with respective substrates . Later the beds were placed in room temperature for 15 to 18 days and after 18 days the beds that turned to whitish mycelial growth after complete spawn run were brought to bamboo plantations and tied to the stems of bamboo in the plantations and ensured that the microclimate of 27 to 30° C and relative humidity of 70 to 75 % prevailed. When the humidity dropped the beds were enclosed with wet gunny cloth where ever needed. At this pin head appearance stage, daily water was sprinkled over the beds. The days for Spawn run (DFSR), Days for pin head formation (DFPF), Days for first harvest (DFFH), yield and biological efficiency (%) were recorded in different treatments.

III. RESULTS AND DISCUSSION

The results obtained from utilization of forest residues for mushroom cultivation and attempts on out door cultivation and relevant discussions on these aspects.

3.1 Selection of substrates and screening of mycelial growth of *P.florida* in forest tree leaf substrates under in vitro (liquid medium)

Whole dried leaves from Teak (*Tectona grandis*), Cadamba (*Anthocepahalus cadamba*) , Casuarina (*Casuarina equisettifolia*), Bamboo (*Bambusa bamboos*) , Sisoo (*Dalbergia sisoo*) , Khaya (*Khaya senegalensis*) , Pungam (*Derris indica*) , Populus (*Populus deltoides*), Eucalyptus (*Eucalyptus teretticornis*) were used for screening the mycelial growth under *in vitro* conditions in PDA medium . The results revealed that the mycelial growth was sparse and slow in Teak, Eucalyptus and Populus when used alone or completely and absolutely no growth was observed in substrates from Cadamba , Khaya, Casuarina, Sisoo, Pungam ; where as good growth of mycelium was observed in paddy straw and bamboo when used alone. Hence it was thought that some of the metabolites from the substrates did no influence mycelial growth .

Hence, the substrates *viz*., Teak, Eucalyptus, Bamboo and Populus were selected and when combined with paddy straw (0.05%) gave positive results. Luxuriant and dense

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mycelial growth was observed Teak, Bamboo, Populus, Eucalyptus leaf litter in combination with paddy and paddy straw substrate alone with 0.15g, 0.18 g, 0.2g, 0.18 g and 0.19 g dry weight respectively (Table 1) These findings clearly indicated that these substrates provided necessary nutrients , cellulose and lignin for the mycelial growth of *P.florida*. It may also be assumed that addition of paddy straw to forest tree residues would have triggered the *P.florida* fungi to induce some enzymes initially and then further induction of lignolyticenzymes may be due to the respective substrates.

In our study, the residues of Casuarina, Khaya, Sisoo, and Pungam did not support the growth of mycelium .Reports show that *Casuarina equisettifolia* is used for the treatment of diarrhea, dysentery, stomach and nervous problems; anthelmintic and anti-diabetic properties (Okuda et.al.,1983) and may perhaps contain inhibitory compounds that would have hindered the growth of the *P. florida* fungal mycelium. Similarly, the leaf exudates from Pungam, Khaya and Sisoo would have exudated some compounds that inhibited the growth of *P.florida*.

Interestingly, the leaf litter from Teak,Eucalyptus, Bamboo and Populus supported mycelial growth of *P.florida* . Though reports show that Teak (*Tectona grandis*) leaf contained Juglone compound with antimicrobial activity (Guptha and Singh ,2004) but in our study teak leaf litter in combination with paddy straw supported good mycelial growth (0.19 g) of *P.florida* similar to paddy straw alone substrate.

Table.1: In vitrogrowth of mycelium of P.florida amended with different substrates (Liquid medium)

Substrate	**Mycelial dry weight (g)**
Teak (*Tectonagrandis*) +Ps	0.15
Cadamba (*Anthocepahaluscadamba*) +Ps	0.10
Casuarina (*Casuarina equisettifolia*) +Ps	0.11
Bamboo (*Bambusa bamboos*) +Ps	0.18
Sisoo (*Dalbergiasisoo*) +Ps	0.12
Populus(*Populusdeltoides*)+Ps	0.20
Khaya (*Khaya senegalensis*)+Ps	0.12
Pungam (*Derris indica*)+Ps	0.10
Eucalyptus (*Eucalyptus teretticornis*)+Ps	0.18
Paddy straw (Ps)	0.19

Ps: Paddy straw

3.2 Cultivation of oyster mushroom in selected forest tree leaf substrates.

Based on the above results, leaf litter from Teak, Bamboo, Eucalyptus, Populus and combination of all substrates were tried for cultivation of oyster mushroom *Pleurotus florida* in thatched sheds Cylindrical beds (500 g dry weight substrate per bed)were prepared with paddy straw and leaf litter from Teak, Bamboo, Eucalyptus, Populusin ratio of 1:1. Similarly , Cylindrical beds were prepared with combination of all the substrates (125 g der weight per substrate). Results revealed that among the substrates teak + paddy straw substrate and paddy straw + combination of all substrates supported early spawn running (16.67 days) followed by paddy straw (18.67 days). The Days for Spawn Run (DFSR) was delayed in Paddy straw+ Bamboo, Paddy straw+ Populus, Paddy straw+ Eucalyptus (19.67, 20.0, 20.33 days respectively). Similarly, the days for pin head formation was much early in Teak + Paddy straw substrate and Paddy straw + combination of all substrates (18.0 and 18.67 days) compared to other substrates. Also the days for first harvest (DFFH) started on 20th day in teak + paddy straw substrate and paddy straw + combination of all substrates (20.0 and 20.67 days respectively) itself compared to other substrates which took around 23-24 days (Fig. 1)

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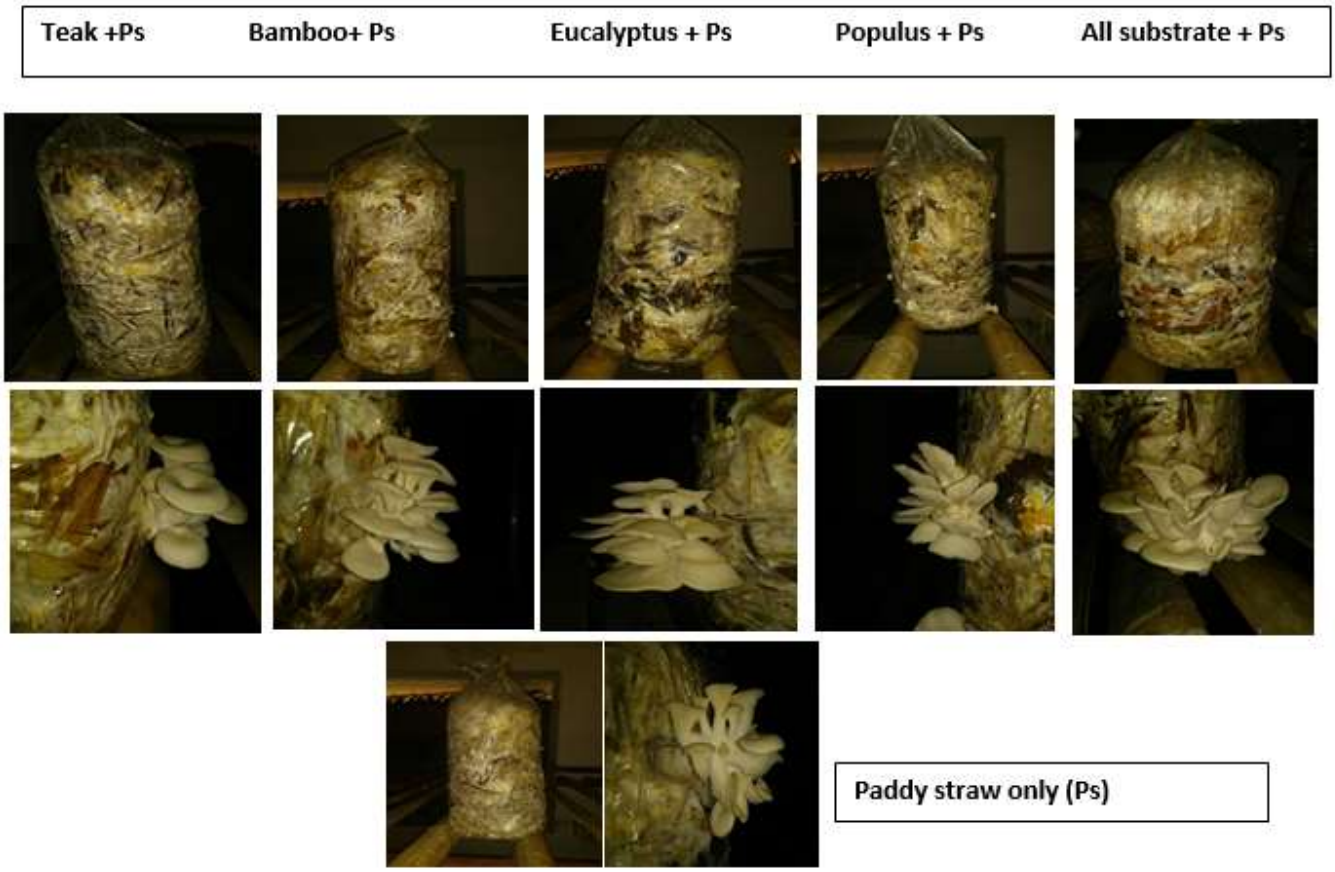

Fig.1: Growth performance of oyster mushroom in different forest tree substrates in combination with paddy straw

Table.2: Yield data of oyster mushroom production using agro forest residues

Substrate	DFSR	DFPF	DFFH	Yield	BE (%)
Teak +Ps	16.67	18.00	20.00	406.67	81.30
Bamboo+ Ps	19.67	21.33	23.00	385.00	77.00
Eucalyptus + Ps	20.33	22.00	24.00	386.67	77.34
Populus + Ps	20.00	21.67	23.67	406.67	81.30
All substrate + Ps	16.67	18.67	20.67	426.67	85.30
Paddy straw (Ps)	18.67	21.00	23.00	470.00	94.00
SEd	1.9833	0.4635	0.5900	15.6466	
CD (%) (P=0.05)	4.4190	1.0327	1.3147	34.8629	

Ps: Paddy strawDFSR: Days for Spawn Run DFPF: Days for Pinhead Formation
DFFH: Days for First Harvest BE: Biological Efficiency (%)

It is assumed that the teak litter enhanced the growth of mycelium of *P.florida* in the presence of paddy straw similar to the results obtained in the liquid medium. Though other substrates *viz*., Eucalyptus did not support the growth of mycelium under lab conditions, however in combination with paddy straw they supported fairly good growth of spawn run and contributedfor pin head formation almost similar to teak and paddy straw substrate. When all the substrates were combined, the nutrients and exudates from the substrates stimulated the growth of mycelium and gave early fruiting. However, the yield was significantly higher in Paddy straw substrate (470g per bed with 500g substrate; 94% bio efficiency) followed by Paddy straw+ combination

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of all substrates (426.67g per bed with 500 g substrate; 85.3 % bioefficiency) (Table 2)

In the present study, Teak + Paddy straw substrate and Teak + Populus though supported good spawn run and recorded yield of 406.67g and 406.67 g per bed with 500 g substrate; 81.3% and 81.3 % bioefficiency respectively. Reduced yield was obtained in Paddy straw + bamboo and Paddy straw + Eucalyptus substrate. Probably, these two substrates did not support moisture content throughout the cropping period due to their waxy nature of leaves and this would have resulted in drying of mycelium and thereby reduction in yield. The results clearly depict that in places with unavailability or shortage of paddy straw, utilization of leaf litter from Teak, Bamboo, Populus, Eucalyptus in combination with paddy straw in the ratio of 1:1 can be used for cultivation of oyster mushroom with bioefficiency of more than 85 per cent. More over, the system fits best in and in agroforestry model to earn additional income by the farmers.

Supporting the present results, leaf litter from *Tectona grandis*, *Terminalia paniculata* and *Xyliaxylocarpa* supported growth of *Pleurotus sajarcaju* with yield ranging from 710 to 960 g bed^{-1} (Maria Florence and Balasundaram, 2000).Reports shows that *Pleurotus florida* can be grown on mango tree substrates especially saw dust individual and in combination with paddy straw with good yield (80% bioefficiency) (Ram Chandra and Deepak Kumar Srivastava,2015) and *P.ostreatus* grew well on mango sawdust with biological efficiency of (Nuruddin Miah *et.al.*, 2016). Tea wastes can be successfully used to cultivate oyster mushroom but it needs to be mixed with sugarcane bagasse for the source of cellulose. Substrate mixture containing tea waste in 40 to 60 % ratio and cotton seed hull can serve as an excellent substrate for oyster mushroom cultivation (Doudou Yang,2015)

Cultivation of oyster mushroom on various agricultural and forestry residue offers high value products with nutritional and medicinal properties. Also, mushroom production provides additional or alternative income to farmers . More over the farmers can be encouraged and provided awareness to develop value added products from mushrooms to fetch higher income

3.3 Outdoor cultivation of oyster mushroom in bamboo plantations

In the present study,.under open field cultivation of oyster mushroom *P.eous* var.APK -1 and *P.florida* among the substrates, paddy straw substrate followed by Paddy straw + bamboo substrate performed better compared to bamboo alone substrate. The days for pin head was early on 11^{th} day in paddy straw substrate followed by 11.5 days in paddy straw+ bamboo substrate. Bamboo alone substrate gave pin heads on 16^{th} day only. Similarly, the yield was maximum in paddy straw substrate (450 g per bed : 90 % bioefficiency) followed by paddy straw+ bamboo substrate (400 g per bed; 80 % bioefficiency). (Fig. 2).

Fig.2: Out door cultivation of oyster mushroom in bamboo plantations

However, bamboo alone when used as substrate gave reduced yield of 350 g/ 500g substrate (70 % BE) when compared to other two substrates which may be due to the poor water retention in the dried leaves of bamboo due to waxy nature. The bamboo substrate in combination with paddy straw gave fairly good yield of 400 g/bed with bioefficiency of 80 per cent .The results clearly show that in bamboo plantations under open field conditions , oyster mushroom can be cultivated as an additional crop by utilizing paddy straw and bamboo litter . The concept of using the vertical space in the main crop is also achieved..Similar results were obtained under open field conditions in bamboo, where *Pleurotus florida* followed by *P.djamor roseus*, *P.platypus* recorded about 90 to 100 % bioefficiency both in paddy straw substrate and in bamboo leaf substrate (Prakasam *et.al.*, 2012)

IV. CONCLUSION

Mushrooms are an excellent source of proteinaceous food to address the problem of malnutrition in young children.. Apart from providing an alternative employment it contributes food security to rural disadvantaged groups especially women and old people. The concept of utilizing the forest tree residues from Teak, Populus, Eucalyptus and Bamboo for mushroom cultivation offers scope for integrating oyster mushroom as a component in integrated agro forestry model.

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